CLAIMS:

1. A method for detecting a hapten in a sample comprising the steps of:

a) providing a sample potentially containing the hapten;

b) providing a pre-determined amount of a first moiety, said first moiety being bound to a signaller and separated therefrom by a first linker, which first moiety is either:

i) a binding partner that specifically binds to the hapten of interest, or

i) the hapten of interest or an analogue thereof;

wherein said signaller is a macromolecule or a nanoparticle providing high mass signal;

c) providing a flow of a) and b) separately or together to an immobilised second moiety, said second moiety being bound to the surface of a sensor and separated therefrom by a second linker, which second moiety is either:

i) a binding partner that specifically binds to the hapten of interest, or

ii) is the hapten of interest or an analogue thereof,

providing that when the first moiety is a binding partner, the second moiety is a hapten or hapten analogue and when the first moiety is a hapten or hapten analogue, the second moiety is a binding partner; and

d) detecting the amount of first moiety bound to second moiety.

2. A method for detecting a hapten in a sample comprising the steps of:

a) providing a sample potentially containing a hapten of interest;

b) providing a pre-determined amount of a binding partner that specifically binds to the hapten of interest, said binding partner being bound to a signaller and separated therefrom by a first linker wherein said signaller is a large protein or a nanoparticle providing a high mass signal;

c) providing a flow of separately or together of a) and b) to an immobilised hapten of interest or an analogue thereof, said hapten or analogue thereof being bound to the surface of a sensor and separated therefrom by a second linker; and

d) detecting the amount of binding partner bound to said immobilised hapten or an analogue thereof.

3. A method for detecting a hapten in a sample comprising the steps of:

a) providing a sample potentially containing a hapten of interest;

b)_ providing a pre-determined amount of the hapten of interest or an analogue thereof, said hapten or analogue thereof being bound to a signaller and separated therefrom by a first linker wherein said signaller is a large protein or a nanoparticle providing a high mass signal;

c) providing a flow of the resultant mixture of a) and b) to an immobilised binding partner that specifically binds to the hapten of interest, said binding partner being bound to the surface of a sensor and separated therefrom by a second linker; and

d) detecting the amount of hapten or analogue thereof bound to said immobilised binding partner.

4. A method for detecting a in a sample using a rapid flow-through inhibition assay format comprising the steps of:

a) Providing a functionalised hapten derivative with a linking group (first linker) between the hapten molecule and its functional group;

b) Providing an immobilised hapten derivative on the surface of an optical biosensor chip;

c) Mixing high molecular weight detecting molecules with sample analytes to form immuno-complexes, and then flow-through of the mixing solution containing excess free antibodies to bind to the sensor surface;

d) Further binding enhancement performed by flowing-through onto the sensor surface with a solution containing a specially designed bio-conjugate, in which by employing a suitable linker (second linker), a moiety to specifically recognise a detecting molecule such as an antibody is linked at one end of the conjugate, and the other end of the conjugate is attached to a large protein or/and a *nano*-particle for high mass signal enhancement;

e) Rapid on-line flow-through regeneration to completely remove detecting molecules such as antibodies for multiple measurements;

f)_A standard curve prepared from solutions with a series of known analyte concentrations, and the concentrations of analyte in unknown samples are then derived from the standard curve.

5. A rapid flow-through competition method for detecting a hapten in a sample comprising the steps of:

a) Providing immobilised detecting molecules onto the biosensor surface with a linker (first linker) between a bio-material as an attachment intermediate and the detecting molecule;

b) Mixing sample analytes with a hapten conjugate, in which a protein or/and a nano-particle is linked to the hapten molecule with a linker (second linker) and having a *nano*-distance (nm) between the protein/*nano*-particle and the hapten molecule to reduce steric hindrance;

c) Flowing through the mixture of hapten conjugate and sample analyte solution onto the sensor surface for binding competition to limited detecting molecules such as antibodies on the surface of the sensor;

6. A method as claimed in any one of claims 1-5 wherein the hapten is selected from the group comprising carbohydrates, polynucleotides, steroids, steroid analogues, polypeptides, drugs, neurotransmitters, hormones and toxins.

7. A method as claimed in claim 6 wherein the hapten is a steroid.

8. A method as claimed in claim 7 wherein the steroid is progesterone.

9. A method as claimed in any one of claims 1-7 wherein the binding partner is selected from antibody molecules and fragments of antibody molecules retaining hapten-binding ability.

10. A method as claimed in any one of claims 1-9 wherein the surface is a surface of an optical biosensor chip.

11. A method as claimed in any one of claims 1-10 wherein the hapten is a steroid and binding of the hapten to the linker occurs at the 4-position of the A-ring structure.

12. A method as claimed in any one of claims 1-11 wherein the hapten is progesterone.

13. A method as claimed in any one of claims 1-12 wherein the first linker and second linker are each independently 10 to 50 atoms in length.

14. A method as claimed in any one of claims 1-13 wherein the first linker and the second linker are independently selected from (a) a carbon-based chain; (b) a carbon-chain containing one or more heteroatoms; (c) a

carbon-chain with substituted groups; (d) an amino acid chain, amino acid fragments incorporated into the chain, or multiple amino-acid fragments chain by homologation; (e) an oligoethylene glycol or a polyethylene glycol chain; (f) a chain having one or more sites of unsaturation such as alkenyl; and (g) a nucleic acid chain; or (h) a polysaccharide chain.

15. A method as claimed in any one of claims 1-14 wherein the hapten is a steroid and the linker between steroid and the surface is an oligoethylene glycol or a polyethylene glycol chain.

16. A method as claimed in any one of claims 1-15 wherein the signaller is a nanoparticle.

17. A method as claimed in any one of claims 1-16 wherein the signaller is an immunogold particle.

18. A SPR-based immunoassay format method comprising the steps:

(a). chemically immobilising a hapten or hapten conjugate onto the optical biosensor surface through a linker molecule (the second linker) with or without using a hapten attachment intermediate,

(b). mixing a fixed concentration of a binding partner - (the first linker)-nanoparticle conjugate in buffer with each of a series of standard free solution or a sample hapten solution and incubating for a few minutes,

(c). injecting the above mixture or the remaining binding partner in equilibrium solution onto the hapten - biosensor surfaces, and measuring binding partner responses,

(d). injecting regeneration buffer onto the biosensor surface to remove binding partner-(the first linker)-nanoparticle conjugate,

(e). plotting concentrations of free hapten versus average response (RU) of binding partner -(the first linker)-nanoparticle conjugate to provide an assay standard curve from which determining the concentration of unknown sample hapten when using the same method.